

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph on page 13 starting at line 21 with the following text:

Figure 1B shows representative flow cytometry plots of gene targeting. GFP positive cells were quantitated in region “R2” as depicted in the ~~left~~ right flow plot. It shows 293/A658 cells after transfection with Sce expression plasmid alone. There are no GFP positive cells. The ~~middle left~~ plot, “S-GT,” shows 293/A658 cells after transfection with RS2100 alone. The two GFP positive cells are circled and represent spontaneous gene targeting events. The ~~right middle~~ plot, “DSB-GT,” shows 293/A658 cells after co-transfection with RS2100 and CBA-Sce. In this plot there are numerous GFP positive cells in region R2.

Please replace the paragraph on page 14 starting at line 1 with the following text:

Figure 1C shows gene targeting rates in 293 Cells. The results are shown as both the number of gene targeting events per million transfected cells (“Events/ 10^6 cells”) plus/minus one standard deviation and as an overall rate. The results are shown for four different gene targets. In the “1 bp ~~mutation~~ insertion” target, a nonsense mutation was created in the GFP gene at bp 321 of the coding region that abrogates functional GFP expression. For the “7 bp insertion” target, a 7 bp sequence was inserted at bp 327 of the GFP coding region. The gene target for the “35 bp insertion” was A658 and the target for the “66 bp insertion” was QQR8 (schematized in Figure 3A). The row labeled “Sce” shows whether Sce was co-transfected or not. The column labeled “Fold Stimulation by Sce Induced DSB” was the stimulation of the gene targeting rate on target A658 induced by expression of Sce.

Please replace the paragraph on page 16 starting at line 14 with the following text:

Figure 4 demonstrates the sequence of the human β -globin gene surrounding the codon mutated (~~in bold~~) to cause sickle cell anemia. Depicted are two pairs of potential chimeric nucleases (HBGZF1 and HBGZF2; HBGZF3 and HBGZF4). The binding sites for the chimeric nucleases are highlighted by being in capital letters.

Please replace the paragraph on page 17 starting at line 8 with the following text:

Figures 12A and 12B: Gene Targeting with GFP chimeric nucleases. A) The sequence of the target sequence in GFP gene and a schematic representation of chimeric nucleases

designed to cleave the GFP gene. The GFP chimeric nuclease target site lies just 5' to the insertion of the I-SceI recognition site ("Sce site"). B) Rate of gene targeting in 293 cells after co-transfection of the indicated nuclease with the repair substrate A767 described in example 1.

Please replace the paragraph spanning pages 46-47, starting at line 20 of page 46 with the following text:

Applicants then explored the variables that regulate the rate of DSB-GT. Applicants found that increasing the amount of substrate (RS2100) transfected increased the rate of DSB-GT until a plateau is reached (Figure 2A). This result demonstrated that gene targeting is dependent on the amount of repair substrate available. Applicants found that increasing the length of homology between the repair substrate and the target linearly increased the rate of DSB-GT (Figure 2B). In these experiments, Applicants kept the amount of 5' homology constant at 290 basepairs (bp) and varied the amount of 3' homology from 500 bp to 3700 bp. This result suggests that while spontaneous gene targeting is logarithmically dependent on homology length (Deng et al., 1992, *Molecular and Cellular Biology*, 12:3365-3371), DSB-GT is linearly dependent on homology length. In either case, increasing the length of homology between the damaged target and the repair substrate increased the frequency with which the cell undergoes gene targeting. Figure 2C shows that the DSB-GT rate was linearly dependent on the amount of PGK-Sce transfected. The DSB-GT rate plateaued, however, when higher amounts of CBA-Sce were transfected (Figure 2C). This data suggests that DSB-GT is dependent on the creation of a DSB to initiate gene targeting but eventually becomes saturated for DSB creation. Applicants found that manipulating the transcriptional status of the repair substrate can affect the rate of gene targeting. Transcribing the truncated repair substrate with a CMV promoter (CMV-RS2100) increased the rate of DSB-GT by 50% (Figure 2D). Just as with RS2100, transfecting CMV-RS2100 into 293-0 cells did not generate GFP positive cells (data not shown). The rate of DSB-GT was highest when Sce expression is driven by the CBA promoter, intermediate with the ~~CMV~~ PGK promoter, and lowest with the ~~PGK~~ CMV promoter (Figure 2E). This result probably reflects the different levels of Sce expression from each promoter. Figure 2E also demonstrates that the rate of DSB-GT can be increased by placing the repair substrate on the same plasmid as the Sce expression cassette rather than co-transfecting two plasmids. The stimulation was lost when the DSB-GT process was at saturation as when the CBA promoter

was used to express Sce. When Applicants optimized the above parameters Applicants achieved gene targeting rates of 3-5% (Figure 2E, CBA promoter).

Please replace the paragraph spanning pages 51-52 and starting on page 51, line 28 with the following text:

Cys2-His2 zinc finger DNA binding domains are modular protein units that can be designed to ~~recognized~~ recognize a wide variety of nucleotide triplets (Wolfe et al., 2000, Annu Rev Biophys Biomol Struct, 29:183-212). For example, using *in vitro* selection techniques, some research groups have designed zinc fingers that can bind with high specificity to all 16 different GNN nucleotide triplets (Liu et al., 2002, J Biol Chem, 277:3850-3856; Segal et al., 1999, Proc Natl Acad Sci U S A, 96:2758-2763). A research group reported a similar characterization of zinc finger domains that could bind all 16 different ANN nucleotide triplets (Dreier et al., 2001, J Biol Chem, 276:29466-29478). Finally, others have proposed a recognition code for all nucleotide triplets (Sera and Uranga, 2002, Biochemistry, 41:7074-7081; Wolfe et al., 2000, Annu Rev Biophys Biomol Struct, 29:183-212). These codes were developed based on the recognition sequences and crystal structures of known zinc finger DNA binding domains. Applicants' prior work established a basis for the rational design of a zinc-finger DNA domain to recognize any triplet.

Please replace the paragraph on page 53 starting at line 3 with the following text:

The sequence of the human β -globin gene surrounding the codon mutated (~~in bold~~) to cause sickle cell anemia is shown in Figure 4. Depicted are two pairs of potential chimeric nucleases (HBGZF1 and HBGZF2; HBGZF3 and HBGZF4). The binding sites for the chimeric nucleases are highlighted by being in capital letters.

Please replace the paragraph spanning pages 55-56, starting at line 19 on page 55 with the following text:

In the GFP gene targeting system we express the human CD8 α gene off a bicistronic transcript that includes the mutated GFP gene. In cell line 293/1004, for example, 95% of the cells are CD8 positive. Applicants found the following sequence 5' GGCGCCCAC CATCGC GTCGCAGCC 3' that spans base pair 441-468 of the human CD8 α gene and fits the inverted repeat consensus described above. Applicants constructed ~~CD8CN1~~ CD8ZF1 to recognize 5'

GTGGGCGCC 3' and ~~CD8CN2~~ CD8ZF2 to recognize 5' GTCGCAGCC 3'. Applicants also constructed a CD8 cDNA knock-out plasmid in which a puromycin resistance cassette is flanked by 440 bases of 5' homology and 220 bases of 3' homology to the CD8 gene (called "CD8 Knockout Plasmid"). Applicants then transfected the CD8 Knockout Plasmid with and without the CD8 chimeric nucleases into cell line 293/1104 and measured the percentage of CD8 positive cells in a population of cells after puromycin selection (Figure 13). Transfecting the CD8 Knockout Plasmid did not change the number of CD8 negative cells from the parent population (5% CD8 negative in both) as expected. After co-transfection of the CD8 chimeric nucleases with the CD8 Knockout Plasmid, over 20% of the cells were now CD8 negative. This shows that chimeric nucleases can stimulate gene targeting in the CD8 α cDNA by stimulating the insertion of the puromycin knockout plasmid into the gene.